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Short-term effect of oral Amoxicillin treatment on the Gut Microbial Community

Composition in Farm Mink (*Neovison vison*)

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One Sentence abstract: Amoxicillin affects the intestinal mucosa-associated bacterial community
in mink despite the very fast gastro-intestinal transit time of these carnivorous animals.

Keywords: mink, *Neovison vison*, carnivore, gut microbiota, amoxicillin, 16S rRNA gene
sequencing

25 **ABSTRACT**

26 It is well documented that antibiotics have pronounced modulatory effects on the intestinal bacterial
27 community of both humans and animals, with potential health consequences. The gut microbiota of
28 mink has however attracted little attention due to low bacterial load and fast gastrointestinal transit
29 time, questioning its relevance. In the present study we hypothesise that oral amoxicillin treatment
30 affects the gut microbiota in mink. This was investigated in a controlled trial including 24 animals
31 of which 12 were treated with amoxicillin for seven days. By applying 16S rRNA gene sequencing
32 we found that the faecal microbiota was markedly altered already after two days of treatment, with
33 a surprising increase in diversity to resemble the feed. The diversity within the mucosa at
34 termination was however reduced, which indicates this compartment as an important colonization
35 site in mink. No impact on blood biochemistry, lipid metabolism, serum amyloid A, vitamins A and
36 E and histomorphology of the gut and liver was found, however, a slight decrease in fat digestibility
37 was observed. We suggest that early life use of amoxicillin in mink production may be
38 counteractive as dysbiosis of the microbiota during infancy is increasingly being recognized as a
39 risk factor for future health.

40
41 **INTRODUCTION**

42 The rich microbial community residing in the intestinal tract of humans and animals is collectively
43 termed the gut microbiota. There is a growing body of evidence manifesting that the function of this
44 gut microbiota is not restricted to general digestion of nutrients in the intestine but also plays a
45 tremendously important role in relation to general health including programming of the immune
46 system especially in early life (Matamoros *et al.* 2013; Albenberg and Wu 2014; Round and
47 Mazmanian 2014). In addition, the gut microbiota has the capacity to synthesize vitamins B and K
48 (LeBlanc *et al.* 2013), deconjugate bile acids (Jones *et al.* 2008) and produce short chain fatty acids

49 and antimicrobial compounds, which overall may limit pathogen colonization and proliferation
 50 (Kamada *et al.* 2013). Perturbation of the intrinsic balance between the hundreds of different
 51 species within this ecosystem is especially profound following exposure to antimicrobial
 52 compounds and it is well established that oral antibiotic treatment may cause large shifts in
 53 intestinal bacterial community composition in both humans (Panda *et al.* 2014), rodents (Tulstrup *et*
 54 *al.* 2015) and numerous other animals representing both vertebrate- (Grønvold *et al.* 2010) and
 55 invertebrate species (Raymann, Shaffer and Moran 2016). The effect of antibiotic treatment on the
 56 gut microbiota is highly dependent on the class and spectrum of the drug in question (Tulstrup *et al.*
 57 2015) and different studies have demonstrated both short- and long-lasting effects of antibiotics not
 58 only on pathogens but also on the resident gut microbiota (Cochetière *et al.* 2005; Jernberg *et al.*
 59 2007; Dethlefsen *et al.* 2008). The extent of the disturbances additionally depends on the dose and
 60 the route of administration as well as the general pharmacokinetic and pharmacodynamic properties
 61 of the drug (Edlund and Nord 2000; Sullivan, Edlund and Nord 2001).

62 To date, very few studies have investigated the effects of antibiotics on the gut microbiota in
 63 carnivorous animals (Suchodolski *et al.* 2009; Grønvold *et al.* 2010) and to our knowledge, no
 64 studies have specifically investigated the impact of antibiotics on farm mink (*Neovison vison*).
 65 Recently, high throughput techniques using 16S rRNA gene sequencing of mucosa associated
 66 intestinal samples obtained from mink has showed that the gut microbiota in adult mink is
 67 commonly dominated by bacteria belonging to the phylum *Firmicutes* (especially the class
 68 *Clostridia*) with few *Bacteroidetes*, but may also contain high numbers of both *Proteobacteria* and
 69 *Fusobacteria* (Bahl *et al.* 2017). The dominance of *Firmicutes* is in accordance with similar studies
 70 sequencing faecal samples from cats and dogs (Handl *et al.* 2011; Garcia-Mazcorro *et al.* 2012),
 71 indicating that mink may be a useful animal model for studying other carnivorous animals as well.
 72 Mink have a relatively short intestinal tract and a fast gastro-intestinal transit time of 3-5 hours

73 (Bleavins and Aulerich 1981; Szymeczko and Skrede 1990). A bacterial density up to 10^8 CFU g⁻¹
74 has been detected in the colon of mink, which is much lower than numbers of bacteria found in
75 many other mammals including dogs (Davis *et al.* 1977; Williams, Buddington and Elnif 1998).
76 Collectively these characteristics could suggest a relatively restricted effect of antibiotics
77 administered orally on the gut microbiota.

78 In Danish mink production, the largest amount of antibiotics is used during the lactation period
79 in May until August (Chriél *et al.* 2012; Jensen *et al.* 2016). At this stage mink kits have a thinner
80 mucus layer lining the gut wall, different enzyme-related properties in the gastrointestinal tract
81 (Hedemann, Clausen and Jensen 2011), and a different microbial community composition
82 compared to adult mink (Williams, Buddington and Elnif 1998) potentially making them more
83 sensitive than adult animals. The broad-spectrum antibiotic, amoxicillin, is the most frequently used
84 antibiotic in mink and is utilized for treatment of various infectious diseases including diarrhoea,
85 urinary tract infections, pneumonia, pleuritis, and abscesses (Pedersen *et al.* 2009; Chriél *et al.*
86 2012). Reports of antimicrobial use in mink in recent years reveal that the oral administration route
87 constitutes 98% of the antimicrobial use measured in Defined Animal Daily Dose (Jensen *et al.*
88 2016). Since oral administration of antimicrobials for mink may involve treatment of either part of
89 or the entire farm, including both sick and asymptomatic animals, there is a need to understand the
90 potential impact of the oral antimicrobial administration on the microbiota of the healthy mink gut.

91 The present study was designed to investigate the induced changes in the bacterial community
92 composition of the gut microbiota as well as changes related to biochemical profile, vitamin A and
93 E levels, lipid metabolism, levels of acute phase reactant serum amyloid A, and histomorphological
94 parameters in the gut and liver following a standard treatment period of 7 days with daily oral
95 amoxicillin administered to clinically healthy growing male mink.

96

97 MATERIALS AND METHODS

98 **Ethical statement**

99 All institutional and national guidelines for the care and use of laboratory animals were followed.

100 The handling of the animals and the experimental procedures were approved by the Danish Animal
101 Experiments Inspectorate (licence no. 2016-15-0201-00965) and all personnel involved in handling
102 and care of the animals were trained for carrying out animal experiments (FELASA certification).

103

104 **Animals and housing**

105 The study was carried out at the Danish Fur Breeders Association research farm, Copenhagen Fur,
106 Holstebro, Denmark from the 12th to the 26th of September 2016. Twenty-four male mink (*Neovison*
107 *vison*) at 4.5 months of age of the colour type Brown (also referred to as colour type Wild) were
108 selected for the study. The mink were all littermates in pairs from 12 different litters with a
109 minimum of 5 kits. The average body mass was $2,809 \pm 300$ g (mean \pm SD) with 584 g as the
110 maximum body mass variance between siblings. All animals had received standard vaccinations
111 (Distemink[®] Vet., Biovet ApS and Biocom-P Vet., Biovet ApS) at the age of 10 weeks and the farm
112 was tested negative for Aleutian Disease Virus. The mink were housed indoors individually in
113 metabolic cages modified from Jørgensen, G. & Glem Hansen (1973) with the possibility for
114 manual ventilation. The average ambient temperature and humidity was $21 \pm 1.8^{\circ}\text{C}$ and $68.3 \pm$
115 4.2% , respectively. All mink were fed 180 g of a diet produced at the local central of feedstuff once
116 a day in the morning and had free access to tap water. The same batch of feed was used throughout
117 the entire study.

118

119 **Experimental design**

120 The study was designed as a parallel group experiment. Mink were allocated in two groups by
121 randomized stratification with one littermate in each group (Fig. 1). Amoxicillin (Octacillin Vet.
122 800 mg g⁻¹, amoxicillin trihydrate corresponding to 697 mg amoxicillin), 20 mg kg⁻¹ was
123 administered orally to the treatment group (AMX) from Day 0 and for 7 consecutive days while no
124 treatment was given to the control group (CON). The antimicrobial compound was mixed in the
125 feed ration. General health checks were made in the morning and afternoon throughout the study.

127 **Gastrointestinal transit time**

128 On Day -4 (relative to the antibiotic intervention) a study of the feed transit time was conducted to
129 verify that the transit time in the gastrointestinal tract (GI-transit time) did not differ between the
130 two groups before the intervention (Fig. 1). All mink received 100 plastic Hama beads (2.5x2.5
131 mm, assorted colours, Malte Haaning Plastic A/S), which have been used as a marker of GI-transit
132 time elsewhere (Hernot *et al.* 2005). The beads were mixed in 50 g of feed in the morning. When
133 the 50 g ration was ingested or after one hour the rest of the daily ration without beads was given.
134 Two hours after beads were ingested the mink started defecating and faeces were collected every 15
135 minutes. Beads were counted after washing the faeces in a sieve under running water. The time
136 lapse until the first bead appeared in the faeces was considered the feed transit time for the
137 individual animal.

139 **Collection of blood and faeces and biochemical analyses**

140 On Days -5, 2 and 7 blood was obtained by puncture of vena cephalica antebrachii and directly
141 transferred to EDTA-coated (plasma) and non-coated (serum) tubes (BD Vacutainer Systems,
142 Preanalytical Solutions, Belliver Industrial Estate, UK). Tubes were centrifuged at 2000 G for 10

143 minutes and isolated plasma and serum was transferred to 0.5 ml eppendorf tubes, respectively.
144 Samples for analysing biochemical parameters in serum as well as parameters related to lipid
145 metabolism in plasma were kept at 5 °C and analysed by Advia[®] 1800 Clinical Chemistry System,
146 Siemens, at Centrallaboratoriet, Department of Veterinary and Animal Sciences, University of
147 Copenhagen, Denmark. Serum samples for analysing acute phase reactant serum amyloid A were
148 stored at -18 °C and analysed by a standard ELISA-kit (Phase SAA assay, Tridelata Development
149 Ltd., Kildare, Ireland) at the National Veterinary Institute at the Technical University of Denmark.
150 Plasma samples for analysing vitamin A and E were stored at -18 °C and subsequently analysed by
151 PerkinElmer Series 200 HPLC System at Foulum, University of Aarhus, Denmark (Jensen, Engberg
152 and Hedemann 1999).

153 On Days -5 and 2 faeces was collected from a sterile plastic drape placed under the cages using
154 disposable forceps and directly transferred to cryovials after voiding (CryoPure Tube 1.8 ml,
155 SARSTEDT, Germany). Samples were kept on dry ice until they were transferred to storage at -
156 80 °C. On Day 7 all mink were euthanized by initial sedation with a mixture of 0.6 ml ketamine
157 (Ketaminol[®] Vet. 50 mg ml⁻¹, MSD Animal Health) and medetomidin (Domitor[®] Vet. 1.0 mg ml⁻¹,
158 Orion Pharma Animal Health) administered intramuscularly followed by an intracardiac injection
159 with 1 ml pentobarbital (Euthanimal 200 mg ml⁻¹, corresponding to 182 mg ml⁻¹ pentobarbital,
160 ScanVet Animal Health A/S).

161

162 **Digestibility trial**

163 A digestibility trial was performed on all animals during the intervention period Day 0 – Day 4
164 (Fig. 1). Total faeces from each animal was collected separately once a day in the morning from
165 a clean chute placed under the wire cage. The faeces was transferred to an individual foil tray
166 and stored at -18 °C. In addition, feed not consumed was also collected for each animal

167 transferred to a foil tray and stored at -18°C. Accumulated feed weighing between 20 and 100 g
168 at the end of the trial, was analysed for content of dry matter. Faeces and samples of the feed
169 were analysed for dry matter, protein, fat and ash. Chemical analysis was made in accordance
170 with EU regulations (Commission Regulation 152/09/EC, 2009) at the analysis laboratory at
171 Dansk Pelsdyr Foder A/S. Calculations of the apparent digestibility was made in accordance
172 with existing literature (Ahlstrom & Skrede 1998).

173

174 **Dissection of animals**

175 Dissection was performed *lege artis* immediately after euthanasia. Tissue samples from the liver,
176 the duodenum as well as the colon and the mesenteric lymph node were removed and transferred to
177 10% neutral buffered formalin prior to processing for histology. Intestinal content obtained from the
178 distal colon was collected and stored as described for faecal samples by the use of a disposable
179 forceps. The collection and storage of mucus (and associated bacteria) was done as previously
180 described (Bahl *et al.* 2017). After processing and haematoxylin and eosin staining, liver sections
181 were graded for hepatic steatosis based on a scoring system applied for classification of non-
182 alcoholic fatty liver disease (Brunt 2007), while the intestine and the mesenteric lymph node were
183 evaluated in the software programme ZEN 2.3 blue edition (Carl Zeiss microscopy GmbH, 2011,
184 Göttingen, Germany) after scanning by Zeiss type Axiostar Plus (Carl Zeiss; Göttingen, Germany).
185 For the section of intestine to be evaluated a minimum of five representative areas on each slide
186 were to be present.

187

188 **Extraction of bacterial community DNA**

189 Total community DNA from mucus, faeces, and feed samples was extracted using the MoBio
190 Power Soil®-htp 96 Well Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to

191 the manufacturer's recommendations, with minor modifications as previously reported (Tulstrup *et*
192 *al.* 2015). DNA concentrations were measured with the Quant-iT dsDNA HS kit (Life
193 Technologies).

194

195 **Bacterial community composition and diversity**

196 The bacterial community composition was determined by partial 16S rRNA gene sequencing of the
197 extracted community. DNA amplification of the V3-region was performed following a dual PCR
198 strategy with universal bacterial primers; PBU (5'-CCTACGGGAGGCAGCAG-3') and PBR (5'-
199 ATTACCGCGGCTGCTGG-3') as described previously (Bahl *et al.* 2017). Sequencing of the 16S
200 rRNA gene libraries was performed on the Ion OneTouchTM and Ion PGM platform with a 318-
201 Chip v2. Sequencing was performed by the DTU in-house facility (DTU Multi-Assay Core
202 (DMAC), Technical University of Denmark. Sequencing data were imported into CLC Genomic
203 Workbench (version 8.5. CLC bio, Qiagen, Aarhus, Denmark) and were de-multiplexed trimmed to
204 remove barcodes and PCR primers. Quality filtering (maxee 1.5), dereplication, OTU clustering
205 (minsize 2), chimera filtering (RDP_gold database), mapping of reads to OTUs (97% similarity)
206 and generation of an OTU table was done according to the UPARSE pipeline (Edgar 2013),
207 generating a total of 2254 non-chimeric OTUs. Taxonomy was assigned using the RDP
208 multiclassifier ver. 2.10.1 with a confidence threshold set to 0.5 recommended for sequences shorter
209 than 250bp (Wang *et al.* 2007). A phylogenetic tree was created with FastTree based on PyNAST
210 alignment of representative OTU sequences with an archaea (*Methanosarcina*) sequence added as
211 outgroup for rooting. In QIIME (Caporaso *et al.* 2010), the OTU table was filtered to exclude OTUs
212 classified as Cyanobacteria/Chloroplast and to exclude OTUs with average relative abundance
213 below 0.005% of the total community (Bokulich *et al.* 2013), resulting in 377 OTUs. Relative taxon
214 abundances and alpha diversity (Observed OTUs and Shannon diversity index) and beta diversity

215 based on unweighted UniFrac distances were calculated in QIIME (core_diversity_analyses.py),
216 with the sequencing depth rarefied to 4,700 sequences per sample. Sequencing data are deposited in
217 the NCBI Sequence Read Archive with the accession number SRP110733.

218

219 **Data handling and statistics**

220 Statistical analysis was performed in GraphPad Prism (version 7.0b; GraphPad Software Inc., La
221 Jolla, CA). Differences between groups were assessed by paired t-test, unpaired Student's t-test or t-
222 test with Welch correction if data had unequal variances. For non-parametric data, Mann-Whitney,
223 Wilcoxon or Fischer's exact test were used as appropriate. Levels of biochemical blood parameters
224 and vitamins were compared between groups on separate days (Day -5, Day 2, and Day 7) as well
225 as through a trend analysis comparing differences between the sampling days. Levels of acute phase
226 reactant serum amyloid A, and parameters related to lipid metabolism were compared between the
227 two groups at the end of the study (Day 7). Statistical significance threshold was set at $P < 0.05$.
228 Identification of differentially abundant bacterial taxa based on 16S rRNA gene sequencing was
229 performed by permutation based tests adjusting for multiple comparisons, with a false discovery
230 rate threshold $q = 0.05$ (Pike 2011).

231

232 **Results**

233 **Effects on the host animals**

234 No morbidity or mortality was recorded during the animal experiment and no gross pathological
235 findings were recorded in necropsy procedures.

236 At Day -4 prior to the intervention, no differences were found in gastro-intestinal transit time
237 between the CON group and the AMX group (2.9 ± 0.65 and 3.1 ± 0.98 h, respectively) (Fig. 2A).

238 Generally, mink in both groups lost body mass during the study (Day -5 to Day 7), but no

239 difference was seen between groups (Table 1) and no significant difference in feed intake was
240 observed during the intervention (Fig. 2B) or before treatment (data not shown).

241 In the present study, no apparent changes in histomorphological parameters related to neither the
242 duodenum nor colon were seen between the two groups after the treatment (Table 1). Furthermore,
243 no reaction in the mesenteric lymph nodes was recorded, and no difference in grading of hepatic
244 steatosis was present between groups (data not shown).

245 Treatment with amoxicillin for 7 days had no significant influence on uptake of fat-soluble
246 vitamins A and E, the level of acute phase reactant serum amyloid A, nor parameters related to
247 lipolysis e.g. triglycerides and non-esterified fatty acids (data not shown), however, significantly
248 higher levels of β -hydroxybutyrate was found in the AMX group on Day 7 (Fig. 2C). There were no
249 differences between groups in the parameters included in the biochemical profile (data not shown).

250 A slight increase in digestibility of fat was seen in the group of mink receiving amoxicillin when
251 compared to the control group during the treatment period (Fig. 2D-F). Data from one control mink
252 in the digestibility trial was excluded from the study because of extremely abnormal values.

253

254 **Effects on the microbiota**

255 On Day -5 before the treatment period, no differences in the community composition of the faecal
256 microbiota between the CON and AMX groups were observed (Fig. 3 and 4). Furthermore no
257 differences in alpha diversity indices were found between groups before the intervention (Fig. 5)
258 and also no separation of groups according to beta diversity was seen on Day -5 as assessed by
259 PCoA analysis of unweighted UniFrac distances (Fig. 6). After two days of amoxicillin treatment
260 (Day 2) the number of observed species in the AMX group was significantly higher than in the
261 CON group ($P<0.05$), and the Shannon index was also higher although not significantly (Fig. 5A-
262 B). Principle coordinate analysis of beta diversity showed that faecal samples obtained from

263 amoxicillin treated animals on Day 2 appeared to cluster together with feed samples and separate
264 from the control animals (Fig. 6B), which is further supported by community composition
265 histograms, where samples from the AMX group Day 2 and Feed samples appear very similar (Fig.
266 3). On Day 2 the relative abundance of numerous bacterial genera were different between the CON
267 and AMX groups (Fig. 4; Table S1, Supporting Information).

268 Analysis of mucus associated bacterial community obtained at the end of the treatment period
269 (Day 7) revealed significantly lower alpha diversity in the AMX group compared to the CON group
270 in terms of both number of observed species and Shannon diversity index (Fig. 5A-B) which
271 appeared consistent with the community composition analysis based on relative abundance of
272 bacterial classes (Fig. 3) as well as the separation of the two groups by PCoA (Fig. 6D). Three
273 bacterial genera belonging to the Clostridia class, namely *Clostridium* sensu stricto, *Anaerococcus*
274 and *Clostridium XI*, were found to have a lower relative abundance in the mucus layer in the AMX
275 group compared to the CON group.

276 No faecal samples were obtained on Day 7; however, intestinal luminal samples were taken with
277 disposable forceps directly from the distal part of the colon during the dissection. These samples
278 appeared similar to mucus samples obtained on the same day in terms of bacterial composition (Fig.
279 3). The same tendency is also seen in beta-diversity (principal coordinate analysis) where intestinal
280 samples from Day 7 and mucosa samples from Day 7 appear to be similar (Fig. 6C-D), which may
281 be due to the fact that the colon contained a sparse amount of luminal content making it difficult to
282 avoid collecting mucus in those samples. For this reason, luminal samples obtained from Day 7 are
283 not included in further analysis, as they are not deemed comparable to faecal sample taken on Day -
284 5 and Day 2.

285

286 DISCUSSION

287 To our knowledge this is the first study to demonstrate that the intestinal microbial community
288 composition of mink, changes considerably during treatment with amoxicillin. However, during the
289 short-term intervention, no changes in parameters included in the biochemical profile, vitamin A
290 and E levels, level of acute phase reactant serum amyloid A, parameters related to the general lipid
291 metabolism nor histomorphological parameters related to the gut nor the liver were observed. The
292 general loss in body mass observed in both groups may be due to a less intensive feeding strategy as
293 well as stress factors during the study period.

294 No difference in GI-transit time was observed prior to the intervention and the community
295 composition in the faecal microbiota between the two groups before the intervention did also not
296 differ (Fig. 2, 3 and 4). Thus, the observed difference in microbiota composition during the
297 administration of amoxicillin is a direct consequence of treatment. The early onset of changes in the
298 gut microbiota after two days of treatment with amoxicillin is consistent with other studies
299 (Cochetière *et al.* 2005). The observed increase in alpha diversity in the faecal microbiota after two
300 days of treatment (Fig. 5) was surprising and not consistent with previous studies in dogs, rats and
301 humans, which all show a significantly decreased microbial alpha diversity after treatment with β -
302 lactams and vancomycin (Grønvold *et al.* 2010; Panda *et al.* 2014; Vrieze *et al.* 2014; Tulstrup *et al.*
303 2015). In the present study we find it very likely that the inhibitory effect of amoxicillin combined
304 with the very fast intestinal transit time in mink and the high bacterial load associated with mink
305 feed (Bahl *et al.* 2017) result in a population structure in faecal samples from amoxicillin treated
306 animals comparable to that found in the feed. As mink feed has a relatively high microbial alpha
307 diversity in terms of both number of observed species and Shannon diversity this results in an
308 apparent increased count of observed species in these animals, which is thus a reflection of
309 allochthonous microbes from the feed ingredients (Savage 1977). Interestingly, within the mucosa
310 the amoxicillin treatment has the opposite effect of reducing the alpha diversity significantly (Fig.

5) which is consistent with other studies (Grønvold *et al.* 2010; Panda *et al.* 2014; Vrieze *et al.* 2014; Tulstrup *et al.* 2015). Within the mucosa (Day 7) a couple of specific genera within the Clostridia were found to be affected by the amoxicillin treatment after correcting for multiple testing, which is supported by the global shift in community composition (Fig. 6D); however, a much more pronounced effect was found at the genera level in the faecal samples obtained on Day 2 (Fig. 4). This supports the notion that genetic material detected in the faecal samples on Day 2 (AMX group) is predominantly from feed-associated bacteria and thus not colonizing microbes. To investigate whether these bacteria are living or dead, a quantification of genetic material could have been applied as performed in other studies (Pérez-Cobas *et al.* 2012). The observed effects within the mucosa-associated bacteria (Fig. 3, 4 and 6) occur despite the very fast transit time in the gastrointestinal tract in mink (Bleavins and Aulerich 1981; Szymeczko and Skrede 1990) allowing limited time for uptake or direct effect of antimicrobials administered in the feed. The observed faecal microbiota in the mink may be partly driven by the resident bacterial community in the mucosa and partly by feed associated bacteria. We therefore speculate that the colonizing microbiota associated with the mucus layer is substantially inhibited by amoxicillin on Day 2 since the composition ingested appears similar to that detected in the faeces. An increased renewal of the colonic mucosa is essential given the fast transit time in the gastrointestinal tract. This is consistent with a recent study showing that a fast transit time is associated with an increased mucosal turnover in the gut and generally related to the bacterial composition and diversity (Roager *et al.* 2016). As there is a link between gastrointestinal-transit time and bacterial composition and diversity, it would have been interesting to have investigated transit time during the treatment period as well.

The elevated level of β -hydroxybutyrate in the AMX group could be caused by reduced appetite in this group even though no significant difference in feed intake was observed (Fig. 2). Fasting is known to induce lipolysis from adipose tissue and the formation of ketone bodies (VanItallie and

335 Nufert 2003); however, no alterations in triglycerides or non-esterified fatty acids were observed as
336 would have been expected in the case of a physiological reaction to fasting (Mustonen *et al.* 2005;
337 Rouvinen-Watt *et al.* 2010).

338 In the present study, no changes were seen in histomorphological parameters related the gut and
339 the liver regarding characteristics of villus-crypt complex, goblet cells, crypt depth, thickness of
340 tunica muscularis or grading of hepatic steatosis, respectively. A previous study with broilers,
341 which are also characterised by having a fast GI-transit time (Ferrando *et al.* 1987), reported
342 increased villus height and improved performance, in terms of significant weight gain after
343 antibiotic growth promoter administration (Sayrafi *et al.* 2011).

344 Maintenance of the host barrier function in the gut is enhanced by the commensal bacterial
345 community, which forms a colonization barrier by e.g. competitive metabolic interactions,
346 occupying intestinal niches and induction of the host immune system (Kamada *et al.* 2013).
347 Furthermore some metabolites, namely butyric acid, from the digestion of oligosaccharides serve as
348 an important energy source for colonocytes (Wang *et al.* 2012) and modulate tight-junctions near
349 the apical surface of epithelial cells (Ohata, Usami and Miyoshi 2005). A compromised barrier
350 function may lead to the invasion of bacteria or bacterial by-product initiating an inflammatory
351 response in the host. Results from the present study, however, did not show any increased levels of
352 acute phase reactant serum amyloid A in the amoxicillin treated animals, which is a marker of
353 inflammation (Jain, Gautam and Naseem 2011).

354 In broilers, antimicrobials have been shown to enhance apparent absorption of alpha-tocopheryl
355 acetate (vitamin E) and fatty acids due to decreased deconjugation of bile acids by *Clostridium*
356 *perfringens* (Knarreborg *et al.* 2004). In the current study, the amoxicillin treated animals showed a
357 lower proportion of *Clostridium* sensu stricto (group containing *C. perfringens*) and a slightly
358 higher digestibility with no effect on protein or carbohydrate digestibility nor the levels of vitamins

359 A and E. According to Hedemann et al. (Hedemann, Clausen and Jensen 2011) the biliary bile acids
360 are exclusively taurine conjugated, but it is not known to which extent they are deconjugated by the
361 gut microbiota.

362 It has been reported for laboratory rodents that antibiotic-induced perturbations of the intestinal
363 microbiota may alter the host susceptibility to enteric infection (Stecher *et al.* 2007; Sekirov *et al.*
364 2008; Endt *et al.* 2010). It would be of great practical relevance for the mink production to
365 investigate whether the observed alterations in the gut microbiota following antimicrobial treatment
366 in mink are associated with increased susceptibility to pathogens. This may especially be relevant in
367 young animals. The current study contributes with useful information regarding experimental
368 design for future studies within this field. Additionally, our findings may contribute to the
369 understanding of gut health in other carnivorous animals subjected to antibiotic treatment.

370

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380

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SUPPLEMENTARY DATA

Additional Supporting Information may be found in the online version of this article:

Table S1. Relative abundance of bacterial groups at different taxonomical levels for individual animals at timepoints Day -5, Day 2, Day 7 as well as Mucus- and Feed samples.

504 **Figure legends:**

505

506 **Fig. 1.** Timeline of experimental period. The acclimatization period was from Day -7 to Day 0 and
507 treatment period from Day 0 to Day 6. All animals were euthanized on Day 7. Blood samples (**B**) as
508 well as faecal samples (**F**) and mucosal samples (**M**) were collected as indicated. Gastrointestinal
509 transit time analysis (**T**) was performed on Day -4 and pathological examination (**P**) was performed
510 during dissection. A digestibility trial (box) was performed from Day 0 to Day 4 during the
511 treatment period.

512

513 **Fig. 2.** Gastrointestinal transit time calculated at Day -4 (A), Percentage feed intake during the
514 intervention calculated from Day 0-7 (B), serum β -hydroxybutyrate concentration determined on
515 Day 7 (C) and digestibility of Fat (D), Protein (E) and Carbohydrate (F) during the intervention
516 (Day 0-4). Boxplots show median and first and third quartile for the control group (CON, n=11-12),
517 and the amoxicillin group (AMX, n=12) with whiskers indicating range. * $P < 0.05$.

518

519 **Fig. 3.** Average bacterial community composition at the class level in faecal samples collected on
520 Day -5, Day 2, and Day 7, as well as in mucus samples obtained on Day 7 for the control group
521 (CON, n=12) and the amoxicillin group (AMX, n=11-12). The bacterial community composition in
522 feed samples collected at the end of the study (Feed, n=5) is also shown.

523

524 **Fig. 4.** Heatmap of z-scores calculated based on average relative abundance within each row for the
525 control group (CON, n=12) and the group receiving amoxicillin (AMX, n=11-12) on Day -5, Day 2
526 and mucus sample (Day 7). The bacterial genera depicted represent above 25% prevalence
527 collectively in all samples. Asterisks indicate significant difference between the control group and
528 the antibiotic treatment group compared at the same treatment time (FDR corrected permutation

based t-test). * $P < 0.05$, ** $P < 0.01$. The left-hand colour bar shows the taxonomic classification of bacterial genera at the class level (Orange: *Actinobacteria*, dark-green: *Bacteroidia*, light-gray: *Flavobacteria*, light-blue: *Bacilli*, blue: *Clostridia*, grey: *Erysipelotrichia*, dark-grey: *Negativicutes*, yellow: *Fusobacteria*, faint-pink: α -*Proteobacteria*, salmon: β -*Proteobacteria*, dark-red: χ -*Proteobacteria*, red: γ -*Proteobacteria* and violet: *Mollicutes*).

534

Fig. 5. Alpha-diversity of the bacterial communities in the faeces samples, mucosal scraps as well as feed samples. Bacterial richness (number of observed OTU's) (A) and Shannon diversity index (B) are shown for faecal samples obtained on Day -5 and Day 2, mucus samples obtained post mortem on Day 7 as well as feed samples obtained at the end of study. Columns show mean values of the control group (CON, n=12), the amoxicillin group (AMX, n=12) as well as feed samples (Feed, n=5) with standard error of the means indicated by error bars. Significant differences between groups are indicated by asterisks * $P < 0.05$ and ** $P < 0.01$.

542

Fig. 6. Beta-diversity represented as principle coordinate analysis based on un-weighted UniFrac distance matrix at Day -5 (A), during the treatment period at Day 2 (B), at the end of treatment at Day 7 (C) as well as mucosa associated microbiota samples collected post-mortem at Day 7 (D). Each dot represents a faecal- or mucus sample from individual animals in the control group (blue, n=12) and amoxicillin group (red, n=12). Feed samples obtained at the end of the study are also included (grey, n=5).

549

550

551 **Tables**

552
553 Table 1. Animal body mass and histomorphological parameters of the intestine.

554

	Control group	Amoxicillin group	<i>p</i> -value
Initial body mass (g) ^a	2789 ± 93	2829 ± 83	0.75
Final body mass (g) ^a	2661 ± 86	2652 ± 73	0.94
<i>p</i> -value	<0.001	<0.001	
Duodenum			
Villus-crypt complex (μm) ^b	1053 ± 85.6 (n=12)	1096 ± 76.2 (n=11)	0.78
Goblet cells ^c	27.1 ± 12.0 (n=12)	28.7 ± 12.3 (n=11)	0.93
Tunica muscularis (μm)	413.3 ± 15.0 (n=10)	424 ± 30.8 (n=11)	0.75
Colon			
Crypt depth (μm)	459.5 ± 25.5 (n=12)	409.9 ± 20.2 (n=11)	0.15
Tunica muscularis (μm)	932.7 ± 96.8 (n=10)	1117 ± 71.4 (n=12)	0.13

567 ^aBody mass measured at day -5 and 7 respectively. ^bVillus-crypt complex measured from villus
568 apex to the base of the crypt. ^cGoblet cells counted on a 400 μm piece of villus apex. Means ±
569 SEM are shown.

570

Figure 1

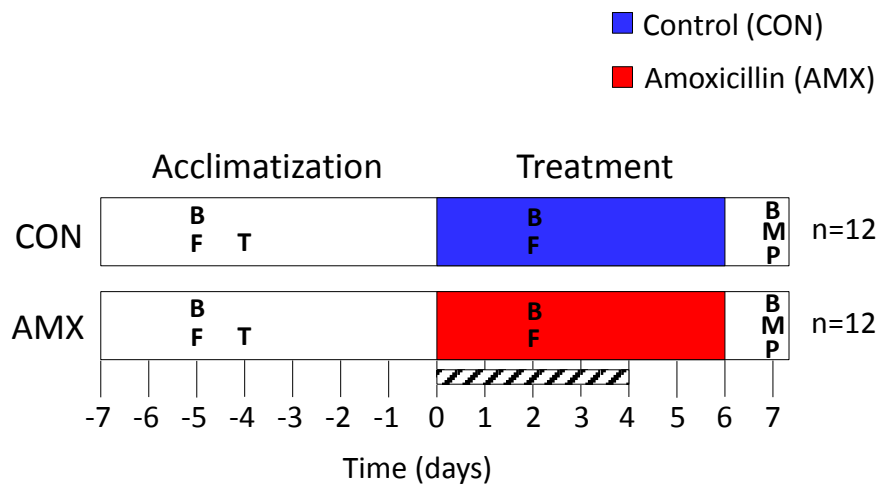


Figure 2

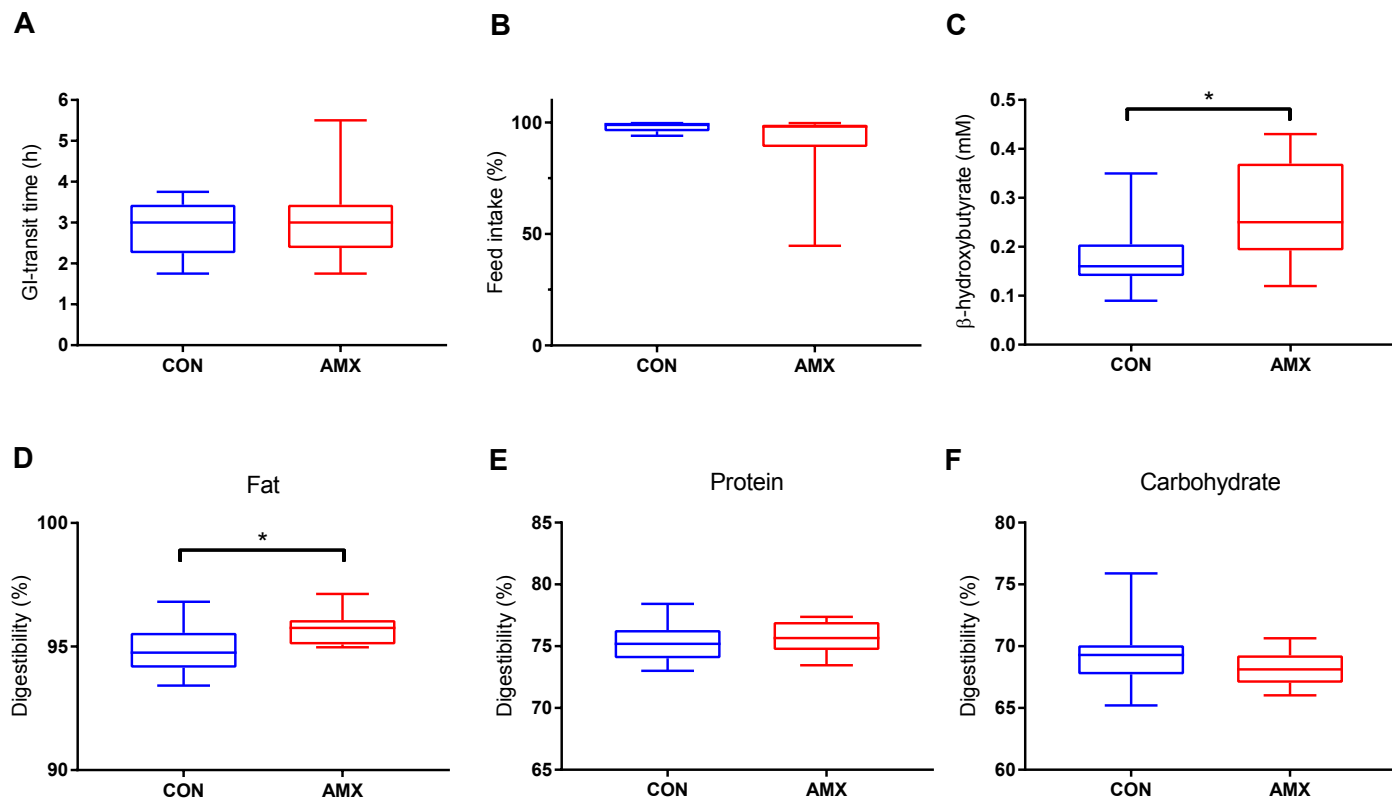


Figure 3

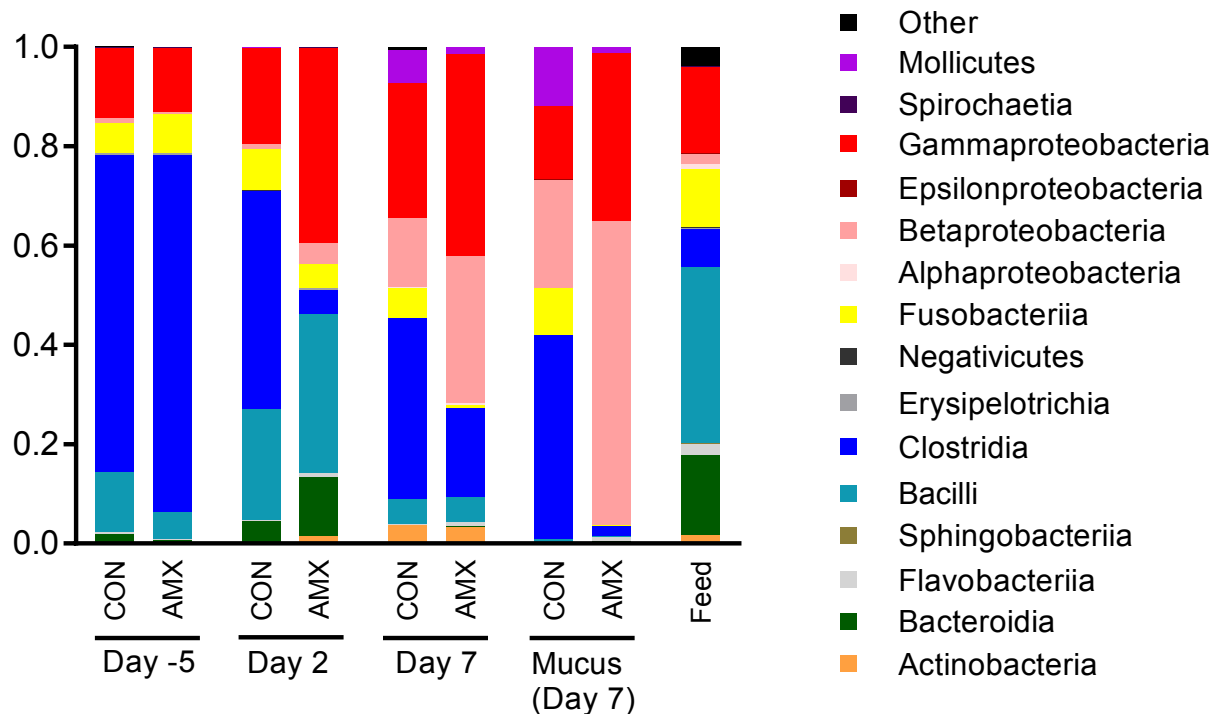


Figure 4

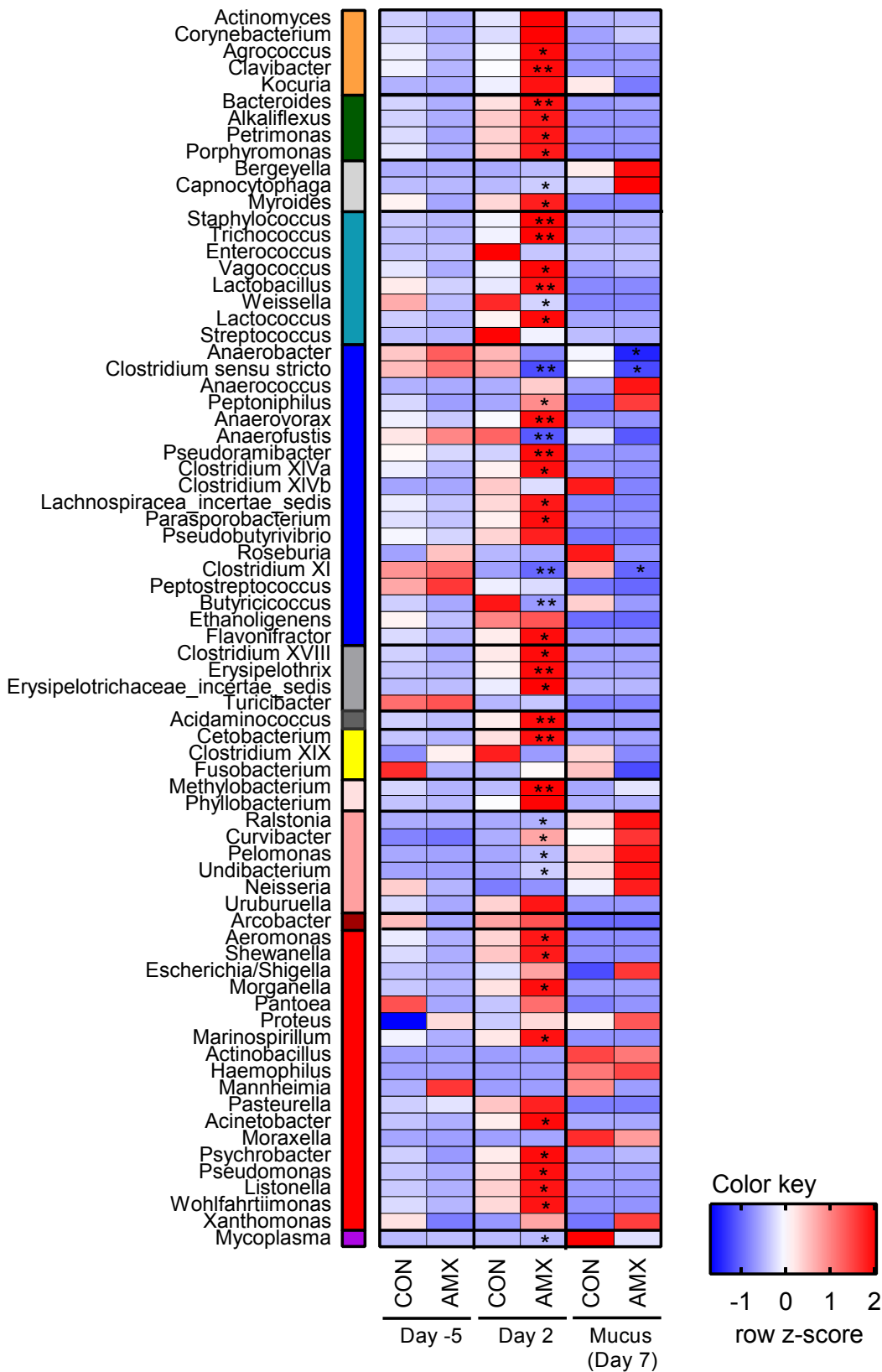
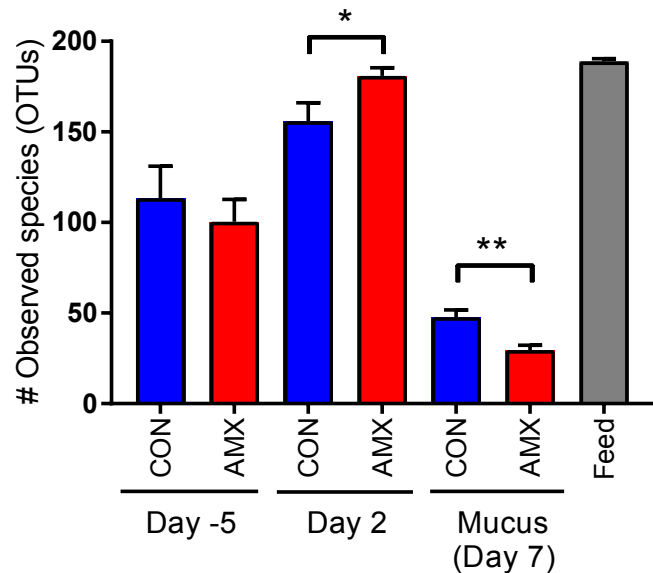


Figure 5

A



B

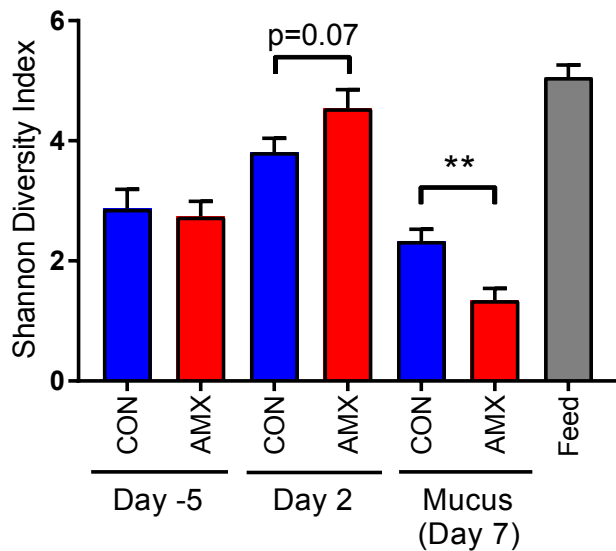


Figure 6

